the cMyBP-CDDD group. Similar results were obtained for maximum force development. As a consequence, LDA was blunted (~40%) in cMyBP-CAAA myocardium. There were no differences in the level of cooperativety as indexed by the Hill coefficient in any group.
Conclusion: Phosphorylated cMyBP-C has been shown to contribute to regulation of cardiac sarcomere function via modulation of the cMyBP-C-actin interaction as well as the disposition of the cross-bridges in relation to the thin filament. Moreover removal of cMyBP-C results in blunted LDA, and a cardiac dysfunction that can be prevented by cMyBP-CDDD but not cMyBP-CAAA. Our data showed that lack of cMyBP-C phosphorylation results in blunted LDA, similar to that found previously in the absence cMyBP-C. We conclude that cMyBP-C phosphorylation modulates myofilament length dependent activation, possibly via modulation of the cMyBP-C interaction with actin.

3912-Pos Board B640
Effect of a High-Salt Diet on the Mechano-Energetics of Left Ventricular Trabeculae Isolated from Dahl Salt-Sensitive Rats
Kenneth Tran[1], June-Chiew Han[1], Carolyn J. Barrett[2], Andrew J. Taberner[3,1], Denis S. Loiselle[1,2], Edmund J. Crampin[4].
[1]Auckland Bioengineering Institute, Auckland, New Zealand, [2]Department of Physiology, School of Medical Sciences, University of Auckland, Auckland, New Zealand, [3]Department of Engineering Science, University of Auckland, Auckland, New Zealand, [4]Electrical and Electronic Engineering, University of Melbourne, Melbourne, Australia.
Dahl salt-sensitive (SS) rats were weaned at 3 weeks of age onto either a high salt diet (4% NaCl) or a normal diet (0.4% NaCl) until termination at 12 weeks of age. Blood pressure measurements, recorded using implanted telemeters, showed a hypertensive response in the high-salt group (MAP = 137 ± 6 mmHg) compared to the normal diet group (MAP = 119 ± 1 mmHg). Compensated hypertrophy was seen in the high-salt group where the wet weights of the hearts were heavier (1.88 ± 0.03 g versus 1.50 ± 0.05 g) and the LV walls were thicker (5.56 ± 0.14 mm versus 4.48 ± 0.12 mm). To investigate the effects of the high salt diet on the mechano-energetics of the heart tissue, trabeculae from the left ventricle were isolated and transferred to a work-loop calorimeter where force production, length change and heat output were simultaneously measured. The experiments were performed at 32°C and the trabeculae were stimulated at 3 Hz. Preliminary results show that there are no statistically significant differences in the peak active stress (62 ± 6 kPa versus 52 ± 6 kPa), peak work (1.67 ± 0.21 kJ m^{-3} versus 1.32 ± 0.16 kJ m^{-3}) or peak mechanical efficiency (16.2 ± 1.1% versus 13.7 ± 1.0%) between the high-salt and normal diet groups, respectively. Hence, despite evidence of hypertension and hypertrophy in the hearts of SS rats fed a high-salt diet, there appear to be no significant differences in the mechano-energetic performance at the tissue (trabecula) level. The data from our experiments are used to parameterize thermodynamically constrained models of cross-bridge kinetics and whole-cell bioenergetics to determine the relationships between model parameters and key experimentally-determined outputs such as work and efficiency.

3913-Pos Board B641
Oxygen Consumption of Brown Adipose Tissue (BAT) and Skeletal Muscle is Inversely Related
Cecilie Sjåland[1,2], Jan Magnus Aronsen[1,2], Madelene Ericsson[3], Hong Qu[4], Øyvind Ellingsen[3], Ivar Sjaastad[1,2], Geir Christensen[1,2], Kristin B. Andersson[1,2], **Per K. Lunde**[1,2], Ole M. Sejersted[1,2].
[1]Institute for experimental medical research, Oslo, Norway, [2]Center for Heart Failure Research, University of Oslo, Oslo, Norway, [3]Department of Circulation and Medical Imaging, Norwegian University of Science and Technology, Trondheim, Norway, [4]Centre for Molecular Biology and Neuroscience, Department of Anatomy, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway.
During exercise, the muscles' energy demand increases with increasing work load. In humans, the relationship between work load and oxygen uptake is linear until the maximal oxygen uptake (VO_{2max}) is reached and higher exercise intensity requires additional anaerobic energy supply. VO_{2max} is thought to reflect the maximum oxygen transport capacity of the cardiovascular system.
We show that at room temperature normal mice could triple running speed at 25% inclination after reaching VO_{2max} in spite of very modest increase of anaerobic muscle metabolism. In mice with cardiac dysfunction due to cardiac disruption of the *Serca2* gene (S2KO), VO_{2max} was reduced from week4 to week6 after gene disruption in parallel with progression of cardiac dysfunction. However, S2KO mice maintained maximal running speed at the same the level as the controls. Thus, paradoxically, running economy was better in S2KO than in controls. In S2KO, blood lactate was almost double of that of controls and respiratory exchange ratio was near 1, indicating greater reliance on anaerobic metabolism. However, heat production was lower in S2KO than in controls as reflected by tail temperature. Activity of BAT measured by fluorodeoxyglucose using PET was reduced by 60 ± 7% during running in controls and by 82 ± 3% in running S2KO mice.
In mice, the oxidative metabolism in non-muscle tissue, mainly in BAT, is reduced during exercise to provide more oxygen to the working muscles. This redistribution of oxygen delivery leaves the total VO_2 unchanged over a wide range of exercise intensities. When cardiac output and VO_{2max} are abnormally low, exercise intensity can be maintained since muscles can utilize the oxygen normally used by non-muscle tissue such as BAT. We conclude that oxygen consumption of skeletal muscle and BAT is regulated in a reciprocal way.

3914-Pos Board B642
Optogenetic G_S Activation in Cardiomyocytes Enhances Pacemaker Activity
Philipp Makowka, Tobias Bruegmann, Thomas Beiert, Philipp Sasse.
University of Bonn, Institute of Physiology 1, Bonn, Germany.
Stimulation of G_S-protein coupled receptors leads to an increase of the second messenger cyclic adenosine monophosphate (cAMP). In cardiomyocytes the G_S-signaling cascade is involved in positive regulation of chronotropy and contractility but chronic G_S stimulation can also induce cardiac hypertrophy or arrhythmia.
Experimentally, the G_S-signaling cascade can be activated by ß-receptor agonists but diffusion of drugs does not allow the precise control of location and timing. To overcome these limitations we used the optogenetic protein JellyOp, a directly G_S-coupled, light-sensitive receptor (Bailes et al. PloS One, 2012) to activate G_S-signaling by light.
Illumination of JellyOp expressing HEK 293 cells resulted in elevation of cAMP levels without detectable dark activity. Cardiomyocytes were differentiated within embryoid bodies (EBs) from transgenic mouse embryonic stem cells that express JellyOp under control of the ubiquitous chicken ß-actin promoter. Spontaneously beating EBs were analyzed at day 13 of differentiation by infrared video microscopy. Brief illumination (20 sec, 470 nm, 166.7 nW/mm^2) increased beating frequency to 1239 ± 349% of baseline (n=3) which returned to baseline after termination of illumination. The lowest effective light intensity was of 9.1 nW/mm^2 resulting in frequency acceleration to 428 ± 131% of baseline and the shortest effective illumination was 1 sec. Similar to dose-response-curves of receptor agonists, light-induced frequency acceleration showed a sigmoid dependence on light-intensity with a half maximal light intensity of 33.5 nW/mm^2. Direct comparison showed that the rate of frequency increment was much faster using illumination (13.9 ± 3.1%/s) than using perfusion with the ß-receptor agonist isoprenaline (2.7 ± 1.5%/s) but both stimulations led to a similar response in frequency elevation.
In summary optogenetic JellyOp activation in cardiomyocytes enables the stimulation of the G_S-signaling pathway with high temporal precision and will be useful to investigate temporal and site-specific effects of physiological and pathophysiological G_S-activation.

3915-Pos Board B643
Compartmentation of Camp Signaling in Complex and Simple Cells
Shailesh R. Agarwal[1], Pei-Chi Yang[2], Colleen Clancy[2], Robert Harvey[1].
[1]Dept of Pharmacology, University of Nevada, Reno, Reno, NV, USA, [2]Dept of Pharmacology, University of California, Davis, Davis, CA, USA.
Differences in compartmentation of cAMP signaling were compared in adult rat cardiac ventricular myocytes and HEK293 cells. The freely diffusible Epac2-camps FRET-based biosensor was used to monitor cAMP responses in the bulk cytoplasmic compartment of these cells, while Epac2-MyrPalm and Epac2-CAAX versions of the probe were used to measure subcellular cAMP responses associated with lipid raft and non-lipid raft domains of the plasma membrane, respectively. Stimulating raft associated beta-adrenergic receptors (βAR) or non-raft associated E-type prostaglandin receptors (EPR) elicited markedly different cAMP responses in the two cell types. In HEK293 cells, maximal βAR or EPR stimulation produced saturating cAMP responses in all three domains. In cardiac myocytes, maximal βAR stimulation produced non-saturating responses in all three domains. However, EPR stimulation produced responses that were smaller, transient, and more consistently observed in non-lipid raft and bulk cytoplasmic domains. There were also significant differences in the pattern of basal cAMP activity associated with the different microdomains of the two cell types. Direct inhibition of adenylyl cyclase (AC) activity with MDL12330A (MDL) only produced a decrease in basal cAMP activity in non-lipid raft domains of HEK293 cells. However, MDL inhibited basal cAMP activity in non-lipid raft domains as well in the bulk cytoplasmic compartment of cardiac myocytes. In cardiac myocytes, responses detected in all three locations were significantly more sensitive to inhibition of phosphodiesterase (PDE) activity. However, in HEK293 cells,

responses detected in non-lipid raft domains were more sensitive to direct stimulation of AC activity with forskolin. Computational modeling suggests that AC activity may play a more important role in explaining the compartmentalized responses observed in HEK293 cells, while PDE activity may be more important in cardiac myocytes.

3916-Pos Board B644

Upregulation of α1-Adrenergic Inotropy in Failing Right Ventricle (RV) is Mediated by the α1A Subtype

Patrick M. Cowley[1], Om Makwana[1], Audrey N. Chang[2], Paul C. Simpson[1], James T. Stull[2], Anthony J. Baker[1].
[1]VA Medical Center & Univ Calif San Francisco, San Francisco, CA, USA, [2]UT Southwestern Medical Center, Dallas, TX, USA.

In nonfailing mouse RV, α1-adrenergic receptors (α1-ARs) mediate a negative inotropic effect (NIE). We reported that α1-AR inotropy in heart failure was dramatically switched to a positive inotropic effect (PIE). The two predominant α1-AR subtypes in heart are the α1A and α1B. However, their inotropic roles in heart failure are unclear.

Goal: Determine the roles of α1A and α1B subtypes in upregulation of α1-AR inotropy in failing RV.

Methods: We used a mouse model of bleomycin-induced RV failure. Bleomycin or saline was instilled into the trachea. Using RV cardiac trabeculae, we assessed in-vitro α1-AR inotropic responses to non-subtype selective agonist phenylephrine (PE, stimulates α1A and α1B subtypes), or subtype-selective agonist A61603 (stimulates only α1A subtype).

Results: Two wk after bleomycin, there was pulmonary fibrosis, pulmonary hypertension and RV failure. For non-failing RV, A61603 caused a NIE (force decreased 48 ± 10%, n=3) similar to the NIE mediated by PE (force decreased 52 ± 12%, n=3). Thus, stimulation of the α1A subtype singly, or together with the α1B subtype, produced a similar NIE. In contrast, for failing RV, stimulation with A61603 caused a switch to a PIE (force increased 134 ± 36%, n=7; $P<0.05$). However, the PIE mediated by PE was much lower (force increased 34 ± 14%, n=7; $P<0.05$). Thus, stimulation of the α1A subtype singly produced a much greater inotropic response versus stimulation of both α1A and α1B subtypes. This suggests that upregulation of α1-AR inotropy in the failing RV was mediated by the α1A subtype, but opposed by the α1B subtype. Preliminary studies suggest a role for myosin light chain kinase in upregulation of α1-AR inotropy in failing RV.

Conclusion: The switch to a PIE induced by α1-ARs in failing RV is mediated by the α1A subtype, not the α1B subtype.

3917-Pos Board B645

Cardiac-Specific Overexpression of FOXO Affords Protection Against Age-Associated Decline in Cardiac Performance in the Drosophila Model

Anna Blice-Baum[1], Guarav Kaushik[2], Meera C. Viswanathan[1], Rolf Bodmer[3], Adam J. Engler[2], Anthony Cammarato[1].
[1]Johns Hopkins University, Baltimore, MD, USA, [2]University of California, San Diego, San Diego, CA, USA, [3]Sanford-Burnham Medical Research Institute, San Diego, CA, USA.

Normal cardiac function declines with age, and a major contributor to heart failure is diastolic dysfunction. The causes of diastolic dysfunction are not yet fully known, although perturbations that affect cardiomyocyte passive mechanics, or stiffness, and calcium handling have been implicated. Here, we have studied the effect of aging on cardiac dysfunction and myocardial stiffening in Drosophila melanogaster, an ideal model for studying senescence due to its short lifespan and ease of genetic manipulation. The transcription factor FOXO, a member of the insulin signaling pathway family shown to mediate an extensive variety of cellular responses in humans, has also been shown to promote general muscle proteostasis and, more specifically, improve cardiac performance following pacing-induced stress in Drosophila. In this study, using high-speed video microscopy and motion analysis, we measured a significant decrease in heart rate and diastolic diameter and increased arrhythmic beating patterns in control fly hearts with age. These changes suggest senescent-related decreases in cardiac output. Furthermore, using an atomic force microscopy-based nanoindentation approach, we determined that control hearts underwent age-related transverse stiffening. Overexpression of FOXO in a heart-specific manner ameliorated these effects as indicated by a lower incidence of arrhythmias, elevated heart rate and increased diastolic diameter with age as well as by affording protection against age-related changes in transverse myocardial stiffness. These data support the hypothesis that increased FOXO activity helps maintain muscle proteostasis in aging hearts. Because aberrant calcium homeostasis in cardiomyocytes may contribute to diastolic dysfunction, we are also interested in evaluating possible cardioprotective effects of FOXO overexpression on calcium handling in aging fly hearts.

3918-Pos Board B646

Myosin Storage Myopathy Mutations Disrupt Myofibrillar Assembly/Stability and Cause Progressive Muscle Degeneration in a Drosophila Model

Meera Cozhimuttam Viswanathan[1,2], William A. Kronert[2], Girish C. Melkani[2], Anthony Cammarato[1], Sanford I. Bernstein[2].
[1]School of Medicine, Johns Hopkins University, Baltimore, MD, USA, [2]Department of Biology and SDSU Heart Institute, San Diego State University, San Diego, CA, USA.

Myosin storage myopathy (MSM) is a congenital disorder caused by dominant missense mutations in the β-cardiac MHC rod and characterized by subsarcolemmal accumulation of β-cardiac myosin that has a hyaline appearance. These mutations map near to or within the assembly competence domain known to be crucial to filament assembly. The mutations disrupt hydropathy or charge of residues in the heptad repeat, altering interactions that stabilize myosin coiled-coil dimers and thick filaments. This potentially disrupts ordered myofibrillar assembly, causing myofibrillar disarray and myosin aggregation. Our *Drosophila* models for MSM make it possible to examine interactions between wild-type and mutant full-length myosins for pursuing mechanistic investigations. We introduced the R1845W, L1793P or the E1883K mutation into a *Drosophila* MHC transgene and expressed each in the indirect flight/jump muscles and in the heart. Our studies show a severe reduction in the flight and jump ability of the transgenic flies in both homozygous and heterozygous states, with an age-dependent worsening of muscle function. Electron and confocal microscopy of the indirect flight muscles of transgenic lines show myofibrillar disarray with large areas of granular/ filamentous inclusions similar to hyaline bodies found in affected humans. Semi-automated optical heartbeat analysis of the mutant heterozygotes shows restrictive cardiac physiology and diastolic dysfunction with evidence of worsening cardiac phenotype with age. Lifespans of the MSM mutants are also reduced in comparison to the transgenic control. Future studies will aim at analyzing *in vitro* filament forming ability of the mutant myosin to determine if defective filament formation and/or instability of the myosin filaments are the basis of MSM. Our model would also potentially help discern if specific chaperones, small molecule chaperone inducers or enhanced autophagy can ameliorate myopathic defects in MSM.

3919-Pos Board B647

Effects of FHC-Related Troponin T Mutations on Proteasome Activity and Half-Life of Troponin T

Jennifer E. Gilda, Ziyou Cui, Aldrin V. Gomes.
NPB, UC Davis, Davis, CA, USA.

Familial hypertrophic cardiomyopathy (FHC) is a genetic disease of the heart muscle that can be caused by mutations in sarcomeric proteins such as cardiac troponin T (TnT), a thin filament regulator of muscle contraction. Results from our lab show that the ubiquitin proteasome system (UPS) is affected in TnT-related cardiomyopathies; in FHC mice expressing the I79N or R278C mutant forms of TnT, changes in proteasome subunit expression and gene expression of proteins in the ubiquitination pathway, increased levels of oxidized proteins, and decreases in proteasome activity in 3 month old I79N mice were observed, suggesting that UPS dysfunction may be an important contributing factor to the pathogenesis of this disease. Mutations in sarcomeric proteins can alter their rates of proteasomal degradation, and increased degradation may lead to proteasome functional insufficiency by competitively inhibiting breakdown of other proteasome substrates. To investigate whether the observed impairment of proteasome function was due to a change in the degradation rate of mutated TnT, the degradation rates of wild-type and mutated (I79N and R278C) TnT were determined in CV-1 cells. The half-life of TnT was not affected by either mutation, suggesting that the effects of these mutations on the proteasome are not due to a difference in the degradation rate of TnT. Experiments to determine the rate of degradation of the FHC mutant F110I are currently being carried out. Overall, our results suggest that the effects of FHC mutations on proteasome function are not due to the mutation directly affecting the proteasome. This work was supported by NIH Grant HL096819.

3920-Pos Board B648

Mirna-448 is a Precursor of Ros-Derived Dystrophic Cardiomyopathy

Sergii Kyrychenko, Viktoriia Kyrychenko, Yoshiyuki Ikeda, Junichi Sadoshima, **Natalia Shirokova**.
Rutgers News Jersey Medical School, Newark, NJ, USA.

NAD(P)H oxidases (NOXs) are of one the major sources of ROS in heart. Recently we reported that NOX2-mediated oxidative stress drives the development of cellular phenotype of cardiac dystrophy. Here we investigated the role of miRNAs in upregulation of NOX2 gene expression. Initial screening with a microRNA target prediction on-line database identified a number of microRNAs that are potential regulators of NOX2 genes. Following qRT-PCR screening of these microRNAs showed a drastic 10-fold down-regulation of